BIOLABO
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MANUFACTURER:
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HDL-CHOLESTEROL Direct Method

Reagent for quantitative determination of HDL-Cholesterol in human serum or plasma

REF 90206 (200-250 Tests)	R1: 1 x 60 mL	R2: 1 x 20 mL
REF 90406 (400-500 Tests)	R1: 2 x 60 mL	R2: 2 x 20 mL
REF 90426 (2000-2500 Tests)	R1: 4 x 150 mL	R2: 4 x 50 mL
Enclosed in each Kit REF 95506:	R1: 1 x 2 mL	R2: 1 x 5 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (3)

The principal role of high density lipoproteins (HDL) in lipid metabolism is the uptake and transport of cholesterol from peripherical tissues to the liver through a process known as reverse cholesterol transport. Low HDL cholesterol levels are strongly associated with an increased risk of coronary heart disease and coronary artery disease. Hence, the determination of serum HDL-Cholesterol is a useful tool in identifying high-risk patients. Increased Total Cholesterol/HDL-Cholesterol ratio is significant of an increased risk of atherosclerosis.

PRINCIPLE

Accelerator selective detergent methodology.
Direct method, without specimen pre-treatment.
During the first phase, LDL, VLDL particles and Chylomicrons generate free Cholesterol, which through an enzymatic reaction, produce Hydrogen peroxide. The generated peroxide is consumed by a peroxidase reaction with DSBmT yielding a colourless product.

During the second phase, specific detergent solubilises HDL-Cholesterol. In conjunction with CO and CE action, POD + 4-AAP develop a coloured reaction which is proportional to HDL-Cholesterol concentration. The absorbance is measured at 600 nm.

LDL = Low density lipoproteins
VLDL = Very low density lipoproteins
CO = Cholesterol Oxidase
4-AAP = 4-Aminoantipyrine
DSBmT = N,N-bis (4-sulphobutyl)-m-toluidine-disodium
HDL = High density lipoproteins
POD = Peroxidase
CE = Cholesterol Esterase
AAO = Ascorbate OxIdase

REAGENTS COMPOSITION

Vial R1 **ACCELERATOR**

Good's Buffer		
CO	< 1000	UI/L
POD	< 1300	ppg UI/L
DSBmT	< 1	mmol/L
Accelerator	< 1	mmol/L
Preservative	< 0.06	%

Vial R2 **SELECTIVE DETERGENT**

Good's Buffer		
CE	< 1500	UI/L
4-AAP	< 1	mmol/L
Detergent	< 2	%
Stabiliser	< 0.15	%
AAO	< 3000	UI/L
Preservative	< 0.06	%

REF 95506 **BIOLABO HDL / LDL / CK MB CALIBRATOR**
Vial R1 (lyophilisate): 1 x 2 mL Vial R2 (diluent): 1 x 5 mL
See enclosed Batch specific Package Insert

REAGENTS PREPARATION

Reagents are ready for use.

STABILITY AND STORAGE

Store at 2-8°C well recap in the original vial and away from light

- When used and stored as described in the insert, unopened reagents and calibrator are stable until expiry date stated on the label.
- Once opened, when free from contamination, reagents R1 and R2 are stable at least for 3 months at 2-8°C, 24 h at room temperature and 30 days on board in refrigerated analysers.
- Once reconstituted, REF 95506: refer to package insert (batch specific)

Discard any reagent if cloudy or if reagent blank at 600 nm > 0.050.
This kit should be refrigerated during transport.

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- For further information, Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

SPECIMEN COLLECTION AND HANDLING (4)

Specimens should be collected after 12 h–14 h fasting.

Plasma: collected on EDTA or lithium/sodium heparinate.
Centrifuge and remove plasma from blood cells as soon as possible (within 3 hours).
Serum: Centrifuge and remove serum from blood cells as soon as possible (within 3 hours).

HDL-Cholesterol in specimen is stable for:

- 1 to 3 days at 2-8°C
- 1 month at - 20°C.

INTERFERENCES (5)

Tested concentrations (mg/dL) without significant interference (± 10%):

Direct bilirubin:	60
Total bilirubin:	60
Hemoglobin:	1000
Ascorbic acid:	100
Lipemia (Intralipid®):	1800
Endogen triglycerides:	2000
Gamma-globulins:	5000

This reagent may interfere with the magnesium determination

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with

Made in France

Latest revision : www.biolabo.fr
Revision : 15/12/2011

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment
2. BIOLABO HDL-Cholesterol calibrator REF n° 95406 or any traceable calibrator of human origin.
3. HDL LDL CK-MB Calibrator REF 95506
4. or any traceable calibrator (human origin).
5. HDL LDL CK-MB controls (human origin)
   REF 95516 HDL LDL CK-MB Control Level 1
   REF 95526 HDL LDL CK-MB Control Level 2
6. or any normal and pathological control sera of human origin.

CALIBRATION

- Do not use aqueous calibrator
- Use BIOLABO HDL-Cholesterol Calibrator REF 95406 traceable to CDC reference method (secondary standard HDL-M04).
- Or HDL LDL CK-MB Calibrator REF 95506 traceable to SRM® 1951b (Standard Reference Material®) assayed on CDC (Center for Disease Control)
- Or a calibrator of human origin traceable to reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

It is recommended to calibrate in the following cases:
1. When changing vial of reagent.
2. After maintenance operations on the instrument.
3. When control values obtained are out of range, even after using a new vial of fresh serum.

QUALITY CONTROL

- REF 95516 HDL LDL CK-MB Control level 1
- REF 95526 HDL LDL CK-MB Control level 2
- Or any assayed control sera of human origin referring to the same method (Selective detergent).
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (6)

HDL-Cholesterol	mg/dL	[mmol/L]
Low level (Risk factor)	< 40	< 1.0
High level (Protective factor)	≥ 60	≥ 1.5

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES CHARACTERISTICS (4)

Within run N = 20	*Low level*	*Medium level*	*High level*
Mean mg/dL	33	51	101
S.D. mg/dL	0.26	0.26	0.71
C.V. %	0.8	0.5	0.7

Between run N = 40	*Low level*	*Medium level*	*High level*
Mean mg/dL	33	50	100
S.D. mg/dL	0.43	0.75	1.1
C.V. %	1.3	1.5	1.1

Detection limit: approximately 2.5 mg/dL.

Sensitivity for 100 mg/dL: 0.120 Abs.

Result of study (n = 52) with designated comparison method (DCM):

BIOLABO Mean: 58.3 mg/dL DCM Mean: 56.3 mg/dL
BIOLABO Range: 33.6-133 mg/dL DCM Range: 32-133 mg/dL
BIOLABO = 0.99 (DCM) + 2.81 mg/dL r = 0.996

LINEARITY

The reaction is linear from 2.5 to 200 mg/dL (0.065 to 5.17 mmol/L).
Above, dilute the specimen (1+1) with saline solution and reassay applying dilution factor 2 to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Do not use aqueous calibrator

Let stand reagents and specimens at room temperature.

Set up the instrument to read micro-volumes.	**Blank**	**Calibrator**	**Assay**
Reagent R1	300 µL	300 µL	300 µL
Calibrator		3 µL	
Specimen			3 µL
Mix vigorously, let stand for 5 minutes at 37°C. Record absorbance A1 at 600 nm against reagent blank			
Add	**Blank**	**Calibrator**	**Assay**
Reagent R2	100 µL	100 µL	100 µL
Mix vigorously, let stand for 5 minutes at 37°C. Record absorbance A2 at 600 nm against reagent blank			

Notes:
1. Depending on the instrument specifications, one can modify the above volumes taking into account the same dilution ratio (i.e. R1 240 µL, R2 80 µL, specimen 2.4 µL or 3 µL). Refer to § LINEARITY.
2. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Automatic analyser with bichromatic reading (600 - 700 nm).is recommended.

With manual procedure, calculate **ΔAbs. = (A2 – 0,75 A1)** for assay and calibrator.

Calculate the result as follows:

$$\text{HDL-C} = \frac{\Delta\text{Abs. Assay}}{\Delta\text{Abs. Calibrator}} \times \text{Calibrator concentration}$$

mg/dL x 0.02586 = mmol/L

REFERENCES

(1) *Badimon L. L., Badimon L., Fuester V., Regression of atherosclerotic lesions by HDL plasma fraction in the Cholesterol-fed rabbit, Journal of clinical investigation, (1990), 85, p.1234-1241.*

(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 564-569*

(3) *Gotto, A.M., Lipoprotein metabolism and the ethiology of hyperlipidemia, Hospital Practice, 23 ; Suppl. 1, 4 (1988)*

(4) *Warnick, G. Russel, Wood, Peter D., National Cholesterol Education Program Recommendations for Measurement of High-Density Lipoprotein Cholesterol: Executive Summary, Clinical Chemistry, Vol. 41, No 10, 1427-1433 (1995)*

(5) *National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol No 7, Vol. 6, No 13, (Aug. 1986).*

(6) *Recommandations de l'AFSSAPS sur la prise en charge thérapeutique du patient dyslipémique, p.9 (Mars 2005).*